

09587653

07/11/2006 11:00 FAX 512 536 4598

FULBRIGHT @ JAWORSKI

038/079

AMENDMENTSAmendments to the specification:

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JH 4/2/07

Please replace the paragraph that starts at page/line with the following paragraph.

First, serum (50 μ L) known to contain GBV-B RNA by RT-PCR assay was extracted with Trizol, and the RNA was washed and dried. A synthetic oligonucleotide was then ligated to the 3' end of the viral RNA. The oligonucleotide, AATTCGGCCCTGCAGGCCACAACAGTC (SEQ ID NO:17), which was phosphorylated at the 5' end and chemically blocked at the 3' end, was ligated to the RNA essentially using the method described by Kolykhalov et al. (Behrens et al., 1996). The RNA was initially dissolved in DMSO and the following additions were made: Tris-Cl, pH 7.5 (10 mM), $MgCl_2$ (10 mM), DTT (5 mM), hexamine cobalt chloride (1 mM), 10 pmol oligo and 8 U T4 ligase. The final concentration of DMSO was 30% in a final volume of 10 μ L. The ligation reaction was incubated for 4 or 20 hours at 19° C. 1 μ L of the ligation reaction was used directly to make cDNA, using a primer complementary to the ligated oligonucleotide and the Superscript 2 system, in a final volume of 15 μ L. 1 μ L of cDNA was amplified using the Advantage cDNA system (Clontech) and two additional oligonucleotide primers. These primers included one that was complementary to the ligated oligonucleotide (i.e., "negative sense") and a positive-sense primer located near the 3' end of the reported GBV-B sequence. A product approximately 290 bases in length was obtained, and this was gel purified and directly sequenced. Sequencing was done in both directions using the oligonucleotide primers employed for the amplification; 259 bases that had not been previously reported were identified as fused to the sequence that had been previously described as the 3' terminus of the viral genome.

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